AD-A210 035

REPORT DOCUMENTATION PAGE

1

Unclassified	1b. RESTRICTIVE MARKINGS DTIC FILE COPY
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) NMRI 89-23	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Naval Medical Research 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION Naval Medical Command
6c. ADDRESS (City, State, and ZIP Code) Bethesda, Maryland 20814-5055	7b. ADDRESS (City, State, and ZIP Code) Department of the Navy Washington, D.C. 20372-5120
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Naval Medical Research and Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Bethesda, Maryland 20814-5055	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62770A; PROJECT NO. 3M162770A870; TASK NO. AN-121; WORK UNIT ACCESSION NO. DA317827

11. TITLE (Include Security Classification)
Immune response gene regulation of immunity to Plasmodium berghei sporozoites and circumsporozoite protein vaccines: overcoming genetic restriction with whole organism and subunit vaccines

12. PERSONAL AUTHOR(S) Hoffman SL, Berzofsky JA, Isenbarger D, Zeltser E, Majarian WR, Gross M, Ballou WR

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
journal article	FROM _____ TO _____	1989	4

16. SUPPLEMENTARY NOTATION
Reprinted from: The Journal of Immunology May 15, 1989, Vol.142(10) p.3581-3584

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Malaria; Plasmodium berghei, Protective immunity; T-helper lymphocytes, Genetic restriction, Malaria vaccine; Sporozoite vaccine; Circumsporozoite protein

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Phyllis Blum, Information Services Division	22b. TELEPHONE (Include Area Code) 202-295-2188
	22c. OFFICE SYMBOL ISD/ADMIN/NMRI

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

0022-1767/89/1410-3581$02.00/0
The American Association of Immunologists

Vol. 142, 3581–3584, No. 10, May 15, 1989
Printed in U.S.A.

IMMUNE RESPONSE GENE REGULATION OF IMMUNITY TO *PLASMODIUM BERGHEI* SPOROZOITES AND CIRCUMSPOROZOITE PROTEIN VACCINES

Overcoming Genetic Restriction with Whole Organism and Subunit Vaccines[1]

STEPHEN L. HOFFMAN,[2]*† JAY A. BERZOFSKY,‡ DANIEL ISENBARGER,† ELANA ZELTSER,‡ WILLIAM R. MAJARIAN,§ MITCHELL GROSS,§ AND W. RIPLEY BALLOU†

*From the *Infectious Diseases Department, Naval Medical Research Institute, Bethesda, MD 20814-5055; the ‡Department of Immunology, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100; the †Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; and the §Department of Molecular Genetics, Smith, Kline, and French Laboratories, Swedeland, PA 19406-2799*

We conducted a series of experiments to define Ir gene regulation of the immune response to *Plasmodium berghei* sporozoites and circumsporozoite (CS) protein-derived subunit vaccines. The studies demonstrated that there is no apparent genetic restriction of the capacity to develop protective immunity against a large sporozoite challenge after immunization with irradiation-attenuated *P. berghei* sporozoites; that the Th response to (Asp-Pro-Ala-Pro-Pro-Asn-Ala-Asn)$_n$, the predominant protective B epitope on the *P. berghei* CS protein, is genetically restricted and regulated by Class II genes (I-A^b) and by genes in the Class I region (H-2D^k) or telomeric to this region; and that this restriction can be overcome by immunization with a r protein including the entire *P. berghei* CS protein. The results support the development of full length human CS protein vaccines to take advantage of all potential T epitopes on this protein.

There are currently two major areas of focus in the malaria sporozoite vaccine development field. One is to define further the cellular mechanisms of protective immunity induced by immunization with irradiated sporozoites (1–6), and to develop subunit vaccines that induce comparable immunity (7). Such vaccines have been studied only in animal models. The second is to develop vaccines that induce protective levels of antibodies to the repeat regions of the circumsporozoite proteins of the human and rodent malarias. Thus far, the response of humans to malaria subunit vaccines has been inconsistent and disappointing in regard to the development of protective levels of antibodies (8, 9). Genetic restriction of the Th response to the T cell-dependent, *Plasmodium falciparum*, repeat region T epitope (10–14) may have contributed to the poor immunogenicity of these vaccines.

Sporozoites from human malarias do not infect mice, thus experiments conducted in the rodent malaria model systems have provided the foundation for human malaria sporozoite vaccine development. To refine our strategies for developing human vaccines, we have used *Plasmodium berghei*, a rodent malaria, to define the role of immune response (Ir) genes in the immunologic response to *P. berghei* sporozoites and subunit CS protein vaccines.

MATERIALS AND METHODS

Mice. H-2 congenic mice on the B10 background were purchased from The Jackson Laboratory, Bar Harbor, ME, provided by Dr. David H. Sachs of the National Cancer Institute, Bethesda, MD, or bred at our facility.

Sporozoites. All experiments were conducted with sporozoites of the NK65 strain of *P. berghei*, that were raised in *Anopheles stephensi* and harvested in Medium 199 containing 5% normal mouse serum.

CS[3] protein Ag. The CS protein of *P. berghei* like other CS proteins, has a central repeat region (15, 16). Immunization with the predominant repeat, Asp-Pro-Ala-Pro-Pro-Asn-Ala-Asn (DPAPPNAN) conjugated to KLH has been shown to induce protective immunity against sporozoite challenge (4). We used the synthetic peptide (DPAPPNAN)$_3$ (Peninsula Laboratories, Belmont, CA) for immunizations and for determination of antibody response by enzyme-linked immunosorbent assay (ELISA). In addition a r protein produced in *Escherichia coli*, pMGB2 CS (7), was used for immunizations. pMGB2 CS is composed of all the amino acid residues of the *P. berghei* CS protein, and at the amino terminus, 6 additional residues (Met-Asp-Pro-Trp-Arg-Lys). The first five are contributed by the expression vector (pMG27 NTrm) (7). Because of a unique Dra I site at the initiating methionine (16) in the genomic clone, the codon for a lysine residue was also included (W. R. Majarian, unpublished).

Immunization with irradiated sporozoites and challenge. Mice were immunized with four intravenous doses (tail vein) of irradiation-attenuated sporozoites (1.5×10^4 R, ^{60}Co source). 7.5×10^4, 2.0×10^4, 2.0×10^4, and 2.0×10^4 irradiation-attenuated sporozoites were given at 2-wk intervals. Mice were challenged 2 wk after the last immunization by the intravenous injection of 10^4 normal sporozoites.

Immunization with (DPAPPNAN)$_3$ and pMGB2 CS. The first dose of 75 μg in PBS, pH 7.2, was emulsified in CFA (Difco Laboratories, Detroit, MI), and the second dose of 50 μg and the third dose of 75 μg were emulsified in IFA (Difco). They were administered intramus-

Received for publication October 28, 1988.
Accepted for publication February 22, 1989.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The opinions and assertions herein are those of the authors and are not to be construed as official or as reflecting the views of the U.S. Navy or the naval service at large. The experiments reported herein were conducted according to the principles set forth in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council, DHHS, Publ (NIH) 86-23 (1985).

[1] Work was supported in part by the Naval Medical Research and Development Command, work unit #3M162770A870AF123, and by our contract N00014-83-C-0355.

[2] Correspondence should be addressed to: Dr. Stephen L. Hoffman, NMRI Annex, 12300 Washington Avenue, Rockville, MD 20852.

[3] Abbreviation used in this paper: CS, circumsporozite.

cularly. Control mice for all experiments received adjuvant alone.

IgG Antibodies to CS protein. ELISA was performed as previously described (4), using $(DPAPPNAN)_3$ as the Ag on ELISA plates.

Indirect fluorescent antibody test. Indirect fluorescent antibody test was performed with NK 65 *P. berghei* sporozoites using methods previously described (17). When incubated with normal serum or serum from mice immunized with adjuvant alone there was no fluorescence at a serum dilution of 1/8.

RESULTS

Protection after immunization. All nine strains of congenic mice that were immunized with four doses of irradiated *P. berghei* sporozoites developed some degree of protective immunity against challenge with 10^4 sporozoites (Table I).

Antibody response after immunization with irradiated sporozoites. All nine strains of mice immunized with irradiated sporozoites made antibodies to DPAPPNAN (Table II). This indicated that Th from all strains of mice responded to $(DPAPPNAN)_3$, or that other T cell sites on the CS protein or the sporozoite were providing T cell help for production of antibodies to $(DPAPPNAN)_3$.

Restriction of response to immunization with $(DPAPPNAN)_3$. To determine which strains actually had Th that responded to $(DPAPPNAN)_3$, we immunized eight of the nine strains of congenic mice (all except B10.RIII) with $(DPAPPNAN)_3$. Only B10 and B10.BR mice made antibodies to $(DPAPPNAN)_3$. A class II molecule on Ag-presenting cells, generally I-A or I-E, is required for the interaction of a Th determinant and the ThR. B10 mice were the only b haplotype mice tested, and the only class II molecule expressed by these mice is $I\text{-}A^b$, indicating that in B10 mice this molecule was involved in the regulation of production of antibodies to $(DPAPPNAN)_3$.

TABLE I

Nine strains of congenic mice (see Table II for H-2 alleles) immunized with four doses of irradiated P. berghei sporozoites were protected against challenge with 10^4 P. berghei sporozoites

Strain	Immunized		Naive	
	N	Infected (%)	*N*	Infected (%)
B10.D2	10	0	10	8 (80%)
B10	15	0	15	12 (80%)
B10.BR	9	2 (22%)	10	9 (90%)
B10.M	10	1 (10%)	10	10 (100%)
B10.S	9	2 (22%)	7	7 (100%)
B10.Q	5	0	5	5 (100%)
B10.P	4	1 (25%)	2	2 (100%)
B10.A	5	0	5	4 (80%)
B10.RIII	4	0		
Total	71	6 (8%)	64	57 (89%)

TABLE II

Antibodies to $(DPAPPNAN)_3$, the predominant octapeptide repeat of the P. berghei CS protein, two wk after the fourth dose of irradiated sporozoites.[a]

Strain	H-2 alleles								Antibodies to $(DPAPPNAN)_3$ (ELISA)
	K	A	B	J	E	C	S	D	
B10	b	b	b	b	b	b	b	b	2.1 ± 0.08
B10.BR	k	k	k	k	k	k	k	k	1.9 ± 0.38
B10.D2	d	d	d	d	d	d	d	d	2.2 ± 0.22
B10.S	s	s	s	s	s	s	s	s	1.6 ± 0.46
B10.A	k	k	k	k	k	d	d	d	2.0 ± 0.21
B10.M	f	f	f	f	f	f	f	f	2.2 ± 0.13
B10.Q	q	q	q	q	q	q	q	q	2.1 ± 0.02
B10.P	p	p	p	p	p	p	p	p	2.3 ± 0.14
B10.RIII	r	r	r	r	r	r	r	r	2.4 ± 0.15

[a] The data represent the mean ± SD absorbance in the ELISA at a dilution of 1/50 of pooled sera. Pooled sera from non-immunized mice of the same strains had an absorbance of 0.1 ± 0.07.

B10.BR and B10.A are identical at the I-A and I-E loci ($I\text{-}A^k$ and $I\text{-}E^k$) (see Table II), yet only B10.BR mice produced antibodies after immunization with $(DPAPPNAN)_3$. Since B10.BR and B10.A mice differ only at the I-C class II, the S, and at the class I H-2D loci (see Table II), we expected that the Ir locus, I-C was involved in regulation of presentation of the Th determinant on $(DPAPPNAN)_3$ to the ThR. To determine if this was the case, we immunized B10.AL mice with $(DPAPPNAN)_3$. These mice are identical to B10.BR at all H-2 class II loci ($H\text{-}2^k$), and at the $H\text{-}2K^k$ class I locus, but differ at the $H\text{-}2D^d$ class I locus (Fig. 1). B10.AL mice did not produce antibodies to $(DPAPPNAN)_3$ (Fig. 1), indicating that the Class I H-2D/L molecules or Qa molecules telomeric to H-2 are involved in the regulatory control of the antibody response to $(DPAPPNAN)_3$ in B10.BR mice.

Overcoming the restriction of response to $(DPAPPNAN)_3$. To determine if there were T cell epitopes on the *P. berghei* CS protein that could provide help for production of antibodies to the repeat region in mice that did not respond to immunization with $(DPAPPNAN)_3$ alone, we immunized the 2 responder strains (B10 and B10.BR) and 4 non-responder strains (B10.D2, B10.A, B10.S, and B10.P) with the full length CS protein, pMGB2 CS. All 6 strains of mice produced antibodies to $(DPAPPNAN)_3$. Among B10, B10.D2, and B10.BR mice the antibody response was comparable to or better than that achieved after immunization with four doses of irradiated sporozoites. The absorbance at serum dilutions of 1/100 and 1/400 for pMGB2 CS vs. irradiated sporozoite immunized animals were 2.9 and 2.6 vs. 2.3 and 1.2 (B10), 1.9 and 0.5 vs. 2.3 and 1.2 (B10.D2), and 2.5 and 1.5 vs. 2.3 and 1.2 (B10.BR). The results of an experiment comparing immunization with $(DPAPPNAN)_3$ to immunization with pMGB2 CS in five strains of mice are shown in Table III. IFA titers against *P. berghei* sporozoites correlated with the level of antibodies to

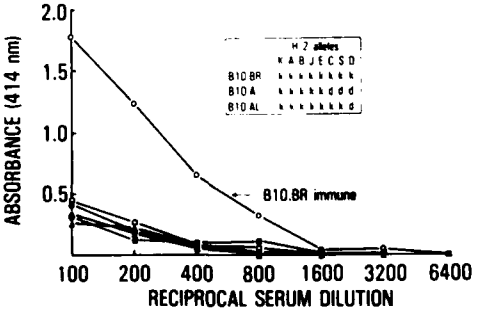

Figure 1. Antibodies to $(DPAPPNAN)_3$ 7 days after the third immunization with $(DPAPPNAN)_3$ in pooled sera (N = 4 or 5); B10.BR (immune ○, controls ●), B10.A (immune □, controls ■), and B10.AL (immune △, controls ▲) mice.

TABLE III

Antibodies to $(DPAPPNAN)_3$ two weeks after the third dose of $(DPAPPNAN)_3$ and pMGB2 CS (full length CS protein)[a]

	Antibodies to $(DPAPPNAN)_3$	
	Immunized with $(DPAPPNAN)_3$	Immunized with pMGB2 CS
B10	1.48 ± 0.167	2.86 ± 0.039
B10.BR	0.41 ± 0.068	2.52 ± 0.081
B10.D2	0.07 ± 0.052	1.92 ± 0.179
B10.S	0.03 ± 0.024	1.10 ± 0.164
B10.A	0.05 ± 0.021	0.56 ± 0.051

[a] The data represent the mean (N = 5) absorbance by ELISA of pooled sera at a dilution of 1/100. Mice immunized with adjuvant in PBS had an absorbance of 0.042 ± 0.017 (Mean ± SD)

$(DPAPPNAN)_3$. They were respectively 1/32 and 1/256 for B10.BR and B10 mice immunized with $(DPAPPNAN)_3$, and were 1/128 (B10.S and B10.A), 1/256 (B10.D2), 1/512 (B10.BR), and 1/1024 (B10) for the mice in Table III immunized with pMGB2 CS.

Challenge of $(DPAPPNAN)_3$ and pMGB2 CS immunized mice with P. berghei sporozoites. Two wk after the third dose of vaccine, the mice whose antibody levels are shown in Table III were challenged by the intravenous administration of 1500 *P. berghei* sporozoites. All of the mice developed an erythrocytic stage *P. berghei* infection.

DISCUSSION

The response of mice immunized with rodent malaria vaccines has been predictive of human response to human malaria vaccines. The observation that immunization with irradiation-attenuated sporozoites protected against sporozoite challenge was made in the *P. berghei* rodent system (18) before being tested in humans (19–22), and the poor protection after immunization with first generation human sporozoite vaccines, that were designed to induce antibody-mediated protective immunity (8, 9), was also predicted by studies in this model system (4).

If there were restriction of induction of a protective cell-mediated response to a whole organism sporozoite vaccine in mice, we would expect great difficulty in developing subunit vaccines to induce a protective cellular response consistently in humans. We therefore designed a study to determine if there was genetic restriction of the capacity of mice to develop cell-mediated protective immunity after immunization with irradiated sporozoites. In a previous study we showed that naive, sublethally irradiated recipients of immune T cells were protected against challenge with 10^4 sporozoites (4). However, mice immunized with a synthetic peptide or an *Escherichia coli*-produced recombinant fusion protein, subunit *P. berghei* CS protein vaccine, despite having much higher levels of antibodies to sporozoites than mice that received immune T cells in adoptive transfer, were protected against challenge with 500, but not 10^3 sporozoites (4). Since this study was designed to determine if there was genetic restriction of the capacity of mice to develop cell-mediated protective immunity after immunization with irradiated sporozoites we challenged with 10^4 sporozoite, a dose expected to overcome antibody-mediated immunity. The finding that all strains of mice developed some degree of protective immunity is encouraging for human vaccine development. None of these mice developed levels of antibodies to the *P. berghei* repeats or whole sporozoites that we consider adequate to protect against such a large sporozoite challenge. We expect that as in the C57BL/6 ($H\text{-}2^b$) (4), Balb/C ($H\text{-}2^d$) (4, 5), and A/J ($H\text{-}2^a$) (6) mice previously studied, the protection was at least in part conferred by cellular immune mechanisms. Since the response to all individual CS protein-derived T cell epitopes studies thus far has been highly restricted (10–13), the finding that all nine strains of mice were protected, implies that there are multiple epitopes involved in this protective, presumably T cell-mediated response. Experiments conducted with a *Salmonella typhimurium* pMGB2 r in $H\text{-}2^d$ mice suggest that there is at least one protective T cell epitope on the CS protein (7). Studies are in progress to define this and other protective T epitopes.

We next undertook to define the pattern of Th response to $(DPAPPNAN)_3$, a protective (4) B epitope on the *P. berghei* CS protein, by assessing production of antibodies after immunization with the peptide. As was the case with the *P. falciparum* protective repeat $(NANP)_n$ (10, 11), the T cell response response to immunization with $(DPAPPNAN)_3$ was restricted. Only two of the nine strains of mice studied produced antibodies to $(DPAPPNAN)_3$ after immunization. In B10 mice the association between $I\text{-}A^b$ class II molecule, Th determinant, and the T cell R required for antibody production appears straightforward. However, the antibody response in B10.BR mice is unusual in that H-2 class II molecules alone do not appear to regulate the response. The results indicate that genes in the class I region, or telomeric to this region regulate the antibody response to this Ag. There is some precedent for class I regulation of an antibody response to equine myoglobin (23), but such regulation is a rare phenomenon.

Genetic restriction of the development of antibodies to a T cell dependent protein antigen can be overcome by addition of carrier molecules to a vaccine. However, in the case of an anti-CS protein vaccine, it would be optimal if priming of Th were accomplished by sporozoite-derived molecules, so that natural exposure to sporozoites could boost vaccine-induced antibodies. Having defined the restriction of response to $(DPAPPNAN)_3$, we immunized mice with pMGB2 CS, a r protein that includes the entire *P. berghei* CS protein. It is likely that the response to any single Th epitope on the CS protein is highly restricted (10–13). Therefore, the demonstration that immunization with pMGB2 CS elicited antibodies to $(DPAPPNAN)_3$ in all six strains of mice studied provides strong evidence that there are multiple Th sites on the *P. berghei* CS protein. An alternative possibility, that the 6 non-CS protein amino acid residues at the amino terminus of pMGB2 CS combined with CS protein derived residues to create a T cell site that provided help in some of the mice that did not respond to $(DPAPPNAN)_3$ alone seems unlikely. It is of interest that immunization with a vaccinia r construct that includes the entire gene encoding the *P. falciparum* CS protein induced antibodies to the repeat regions in a minority of strains of mice studied (24). This incomplete response as compared to that to pMGB2 CS may be due to differences between the CS proteins of the two species of malaria, the immunogens secondary to glycosylation or other post translational modifications, or the processing or presentation of the *E. coli* and vaccinia-produced CS proteins.

Immunization with $(DPAPPNAN)_2$ conjugated to KLH (4), with a r protein including most of the *P. berghei* CS protein (4), and with pMGB2 CS (unpublished) protect against challenge with 500-1000 *P. berghei* sporozoites. The 3 doses of pMGB2 CS used in this study did not protect against challenge with 1500 *P. berghei* sporozoites. The presence of additional CS protein-derived Th epitopes on the *E. coli*-produced protein did not improve the protective antibody response as compared to the $(DPAPPNAN)_2$-KLH vaccine. Nor did this regimen induce the cell-mediated immune response thought to protect mice immunized with *S. tyhimurium* transformed with the pMGB2 plasmid (7). Achieving expression of full

length *P. falciparum* and *P. vivax* CS protein vaccines, analogous to pMGB2 CS, adequate for economical protein purification has been technically difficult. Since the repeat region is the target for antibody vaccines, the approach has been to include non-malaria carriers to provide T cell help for this highly restricted epitope (8, 9, 26). Our data support the development of full length human CS protein vaccines to take advantage of all CS protein-derived T epitopes. Such epitopes may play a role in improving the response to immunization with vaccines designed to produce antibodies to the repeat region of the CS protein, providing sites for boosting of primary antibody responses by natural exposure to sporozoites, and inducing cell-mediated immunity. However, unless Ag presentation and processing can be improved by use of novel adjuvants, carriers, or other methods, the humoral or cellular immune responses induced by immunization with such vaccines may not consistently protect against exposure to intense malaria transmission.

Acknowledgments. We thank Dr. Imogene Schneider, Meegan Dowler, and Mark Haxton for sporozoites; Joe Williams, Rufus Gore, and Mary Leef for technical support; and Dr. David Sachs for some of the congenic mice.

REFERENCES

1. **Spitalny, G. L., J. P. Verhave, J. H. E. Th Meuwissen, and R. S. Nussenzweig.** 1977. *Plasmodium berghei*: T cell dependence of sporozoite-induced immunity in rodents. *Exp. Parasitol. 42:73.*
2. **Chen, D. H., R. E. Tigelaar, and F. I. Weinbaum.** 1977. Immunity to sporozoite-induced malaria infection in mice I. The effect of immunization on T and B cell deficient mice. *J. Immunol. 118:1322.*
3. **Verhave, J. P., Strickland, G. T., Jaffe, H. A., and Ahmed, A.** 1978. Studies on the transfer of protective immunity with lymphoid cells from mice immune to malaria sporozoites. *J. Immunol. 121:1031.*
4. **Egan, J. E., J. L. Weber, W. R. Ballou, M. R. Hollingdale, W. R. Majarian, D. M. Gordon, W. L. Maloy, S. L. Hoffman, R. A. Wirtz, I. Schneider, G. R. Woolett, J. F. Young, and W. T. Hockmeyer.** 1987. Efficacy of murine malaria sporozoite vaccines: implications for human vaccine development. *Science 236:453.*
5. **Weiss, W. R., M. Sedegah, R. L. Beaudoin, L. H. Miller, and M. F. Good.** 1988. CD8$^+$ cells (cytotoxic/suppressors) are required for protection in mice immunized with malaria sporozoites. *Proc. Natl. Acad. Sci. USA 85:573.*
6. **Schofield, L., J. Villaquiran, A. Ferreira, H. Schellekens, R. Nussenzweig, and V. Nussenzweig.** 1987. Gamma interferon, CD8$^+$ cells and antibodies required for immunity to malaria sporozoites. *Nature 330:664.*
7. **Sadoff, J. C., W. R. Ballou, L. S. Baron, W. R. Majarian, R. N. Brey, W. T. Hockmeyer, J. F. Young, S. J. Cryz, J. Ou, G. H. Lowell, and J. D. Chulay.** 1988. Oral *Salmonella typhimurium* vaccine expressing circumsporozoite protein protects against malaria. *Science 240:336.*
8. **Ballou, W. R., S. L. Hoffman, J. A. Sherwood, M. R. Hollingdale, F. A. Neva, W. T. Hockmeyer, D. M. Gordon, I. Schneider, R. A. Wirtz, J. F. Young, G. F. Wasserman, P. Reeve, C. L. Diggs, and J. D. Chulay.** 1987. Safety and efficacy of a recombinant DNA *Plasmodium falciparum* sporozoite vaccine. *Lancet 1:1277.*
9. **Herrington, D. A., D. F. Clyde, G. Losonsky, M. Cortesia, J. R. Murphy, J. Davis, S. Baqar, A. M. Felix, E. P. Heimer, D. Gillessen, E. Nardin, R. S. Nussenzweig, V. Nussenzweig, M. R. Hollingdale, and M. M. Levine.** 1987. Safety and immunogenicity in man of a synthetic peptide malaria vaccine against *Plasmodium falciparum* sporozoites. *Nature 328:257.*
10. **Good, M. F., J. A. Berzofsky, W. L. Maloy, Y. Hagashi, N. Fujii, W. T. Hockmeyer, and L. H. Miller.** 1986. Genetic control of the immune responses in mice to *Plasmodium falciparum* sporozoite vaccine. Widespread non-responsiveness to single malaria T epitope in highly repetitive vaccine. *J. Exp. Med. 164:655.*
11. **del Guidice, G., J. A. Cooper, J. Merino, A. S. Verdini, A. Pessi, A. R. Togna, H. D. Engers, G. Corradin, and P. H. Lambert.** 1986. The antibody response in mice to carrier-free synthetic polymers of Plasmodium falciparum circumsporozoite repetitive epitope is I-A^b-restricted: possible implications for malaria vaccines. *J. Immunol. 137:2952.*
12. **Good, M. F., D. Pombo, I. A. Quakyi, E. M. Riley, R. A. Houghten, A. Menon, D. W. Alling, J. A. Berzofsky, and L. H. Miller.** 1988. *Human T cell recognition of the circumsporozoite protein of Plasmodium falciparum.* Immunodominant T cell domains map to the polymorphic regions of the molecule. *Proc. Natl. Acad. Sci. USA 85:1199.*
13. **Hoffman, S. L., C. N. Oster, C. Mason, J. C. Beier, J. A. Sherwood, W. R. Ballou, M. Mugambi, and J. D. Chulay.** 1989. Human lymphocyte proliferative response to a sporozoite T cell epitope correlates with resistance to falciparum malaria. *J. Immunol. 142:1299.*
14. **Campbell, J. R., F. P. Paleologo, E. D. Franke, S. Ratiwayanto, H. Hadiputranto, L. Kurniawan, R. Wistar, Jr., S. L. Hoffman, B. A. Annis, G. Wasserman, and L. W. Laughlin.** 1988. Immune response of humans to the circumsporozoite protein of *Plasmodium falciparum*: limited T cell response to the immunodominant central repeat region. *Am. J. Trop. Med. Hyg. 39:232.*
15. **Weber, J. L., J. E. Egan, J. A. Lyon, R. A. Wirtz, Y. Charoenvit, W. L. Maloy, and W. T. Hockmeyer.** 1987. *Plasmodium berghei*: cloning of the circumsporozoite protein gene. *Exp. Parasitol. 63:295.*
16. **Eichinger, D. J., D. E. Arnot, J. P. Tam, V. Nussenzweig, and V. Enea.** 1986. Circumsporozoite protein of *Plasmodium berghei*: gene cloning and identification of the immunodominant epitopes. *Mol. Cell. Biol. 6:3965.*
17. **Pacheco, N. D., C. P. A. Strome, F. Mitchell, M. P. Bawden, and R. L. Beaudoin.** 1979. Rapid, large-scale isolation of *Plasmodium berghei* sporozoites from infected mosquitoes. *J. Parasitol. 65:414.*
18. **Nussensweig, R. S., J. Vanderberg, H. Most, and C. Orton.** 1967. Protective immunity produced by the injection of x-irradiated sporozoites of *Plasmodium berghei*. *Nature 216:160.*
19. **Clyde, D. F., V. C. McCarthy, R. M. Miller, and R. B. Hornick.** 1973. Specificity of protection of man immunized against sporozoite-induced falciparum malaria. *Am. J. Med. Sci. 266:398.*
20. **Rieckmann, K. H., P. E. Carson, R. L. Beaudoin, J. S. Cassells, and K. W. Sell.** 1974. Sporozoite induced immunity in man against an Ethiopian strain of *Plasmodium falciparum*. *Trans. R. Soc. Trop. Med. Hyg. 68:258.*
21. **Clyde, D. F., V. C. McCarthy, R. M. Miller, and W. E. Woodward.** 1975. Immunization of main against falciparum and vivax malaria by use of attenuated sporozoites. *Am. J. Trop. Med. Hyg. 24:397.*
22. **Rieckmann, K. H., R. L. Beaudoin, J. S. Cassells, and K. W. Sell.** 1979. Use of attenuated sporozoites in the immunization of human volunteers against falciparum malaria. *Bull. WHO 57(Suppl 1):261.*
23. **Berzofsky, J. A., G. K. Buckenmeyer, and G. Hicks.** 1982. Genetic control of the immune response to myoglobins. VI. Distinct Ir genes for different myoglobins: complementing genes in I-A and H-2D for equine myoglobin. *J. Immunol. 128:737.*
24. **Good, M. F., W. L. Maloy, M. N. Lunde, H. Margalit, J. L. Cornette, G. L. Smith, B. Moss, L. H. Miller, and J. A. Berzofsky.** 1987. Construction of a synthetic immunogen: use of a new T-helper epitope on malaria circumsporozoite protein. *Science 235:1059.*